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RESPONSE OF *CULICOIDES SONORENSIS* (DIPTERA: CERATOPOGONIDAE) TO 1-OCTEN-3-OL AND THREE PLANT-DERIVED REPELLENT FORMULATIONS IN THE FIELD

YEHUDA BRAVERMAN,[1] MARY C. WEGIS[2] AND BRADLEY A. MULLENS[3]

ABSTRACT. The potential attractant 1-octen-3-ol and 3 potential repellents were assayed for activity for *Culicoides sonorensis,* the primary vector of bluetongue virus in North America. Collections using octenol were low, but numbers in suction traps were greater in the high-octenol treatment (11.5 mg/h) than in the low-octenol treatment (1.2 mg/h) or unbaited control for both sexes. Collections using high octenol, CO_2 (~1,000 ml/min), or both showed octenol alone to be significantly less attractive than either of the CO_2 treatments and that octenol did not act synergistically with this level of CO_2. A plant-derived (Meliaceae) extract with 4.5% of active ingredient (AI) (Ag1000), heptanone solvent, Lice free® (2% AI from plant extracts in water), Mosi-guard with 50% *Eucalyptus maculata* var. *citriodora* Hook extract, and *N,N*-diethyl-*m*-toluamide (deet) were applied to polyester–cotton coarse mesh nets and deployed in conjunction with suction light traps plus CO_2. Collections in the trap with deet were 66% lower ($P < 0.05$) than the heptanone and 56% ($P > 0.05$) less than the untreated (negative) control. Relative to deet, collections in the traps with the lice repellent, Ag1000, and Mosi-guard were reduced by 15, 34, and 39%, respectively ($P > 0.05$). The method has promise for field screening of potential repellents before on-animal testing.

KEY WORDS *Culicoides sonorensis,* octenol, carbon dioxide, repellents, sampling

INTRODUCTION

Most *Culicoides* spp. (Diptera: Ceratopogonidae), like many other hematophagous arthropods, are attracted to carbon dioxide (Nelson 1965, Kline et al. 1994), which is now widely used in sampling surveys. Some species respond to lactic acid (Kline and Wood 1988), but more work has been done with 1-octen-3-ol (hereafter called octenol). Octenol showed attractancy to *Culicoides impunctatus* Goetghebuer (Blackwell et al. 1996, 1997) and to *C. furens* (Poey) either alone or together with CO_2 (Kline et al. 1988, 1994; Takken and Kline 1989). In southeastern Queensland, Australia, octenol acted as a synergist with CO_2 for 4 estuarine species of *Culicoides* (Ritchie et al. 1994). The potential activity of octenol against *C. sonorensis* Wirth and Jones, the primary vector of bluetongue virus in North America (Holbrook et al., 2000), has not been evaluated to date.

A few controlled studies have been done to document effects of repellents against *Culicoides* and the duration of their activity on humans (Schreck et al. 1979, Schreck and Kline 1981, Trigg 1996, Trigg and Hill 1996) and poultry (Kitaoka et al. 1965). Most studies compared the repellency of various compounds with that of *N,N*-diethyl-*m*-toluamide (deet) (Schreck and Kline 1981, Magnon et al. 1991) without necessarily documenting the duration of repellency. In the laboratory, a *Eucalyptus*-based repellent prevented *C. sonorensis* from feeding on treated human arms for up to 9 h (Trigg and Hill 1996). Repellents may be effective against a wide range of arthropod disease vectors and are considered to be a strategic means of disease control, whereas vaccines are specific to each disease, and only a few vaccines are available against arthropod-borne diseases (Webster et al. 1991, Gupta and Rutledge 1994). A novel method for preliminary field screening of potential repellents was developed by Braverman and Chizov-Ginzburg (1997), who showed that polyester netting impregnated with a proprietary plant-derived (Meliaceae) preparation, Ag1000, repelled *Culicoides imicola* Kieffer from light traps for 4 h.

To date we lack field information on potential attractants or repellents for *C. sonorensis.* The present study was designed to test the potential activity of octenol and to screen various available plant-derived repellents that might be used to protect animals against *Culicoides*-borne diseases such as bluetongue.

MATERIALS AND METHODS

Octenol studies: The octenol studies were conducted between August 7 and September 2, 1997, adjacent to dairy wastewater ponds at the AK dairy in western Riverside County, California. The dairy cattle were held in pens north of the trap area and no closer than 40–50 m to the traps. The margins of these ponds provided an excellent developmental site for *C. sonorensis.* Experiments were done in two phases. From August 7 to 20, portable suction traps (John W. Hock Company, Gainesville, FL), with the light bulb removed, were set out in a 3 × 3 Latin-square design (9 traps) (Fig. 1). Insects approaching this trap are sucked into a mesh catch bag and retained there by a battery-operated fan. Traps were placed 35 m apart and were suspended from 3.8-liter gray paint cans with the trap entrance

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[1] Kimron Veterinary Institute, PO Box 12, Bet Dagan 50250, Israel.

[2] 17 Sciences Gardens, Edinburgh EH9 1NR, Scotland.

[3] Department of Entomology, University of California, Riverside, CA 92521.

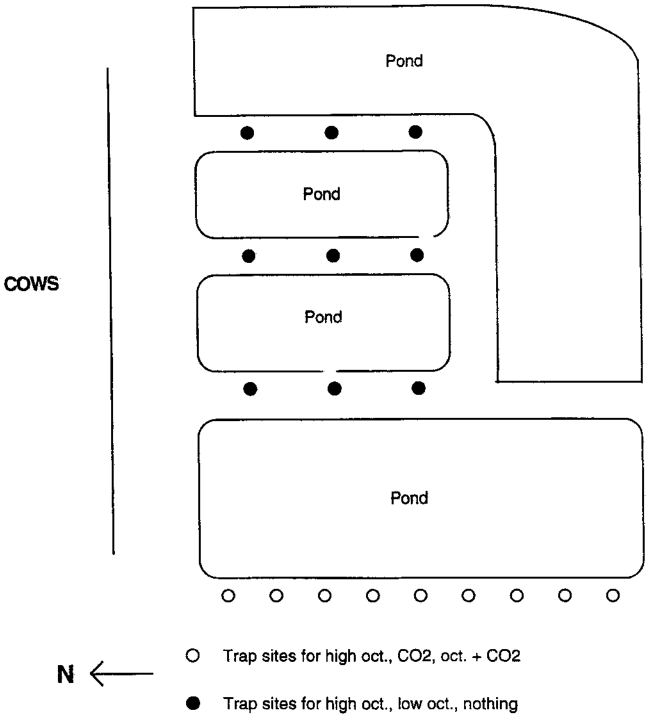

Fig. 1. Diagram (not exact scale) of suction trap locations at AK dairy used for *Culicoides sonorensis* collecting employing octenol only (phase 1) and octenol + carbon dioxide treatments (phase 2). Closed circle traps were separated by 35 m; open circle traps were separated by 20 m.

0.6 m above the ground. Three treatments (3 replications/night) were no-bait (control), a low octenol release rate (1.2 ± 0.6 mg/h), and a high octenol release rate (11.5 ± 1.4 mg/h). Low octenol release rates were achieved by using glass capillary tubes (Blu-tip Caraway Capillary Tubes, Oxford Labware, St. Louis, MO) (75 mm long, 4-mm diameter, 370-μl volume), heat-sealed at one end, with a pipe cleaner inserted and cut flush with the capillary tube mouth. High octenol release rates were achieved using a microreaction-vial method similar to that of Kline et al. (1994), except that the pipe cleaner was doubled through 2 2.7-mm holes drilled in the top of the vial. Release rates were calculated as changes in weight (mg) from just before leaving for the field to just after arriving back at the laboratory the next morning. Weight loss was divided by the number of hours deployed to generate estimates of the average loss in mg/h. These experiments were conducted over 5 nights, deploying the traps approximately 2 h before sunset and retrieving them 2 h after sunrise. Collected insects were counted by sex and recorded.

The 2nd phase of the experiment was conducted from August 28 to September 2. Nine suction traps were placed in a line 20 m apart, on the west side of 1 of the wastewater ponds (Fig. 1). Treatments were randomized within each of 3 blocks along the length of the trap line. The line of traps was placed perpendicular to the prevailing westerly wind to minimize interference from odors drifting downwind. The 3 treatments used were high-level octenol release alone, CO_2 alone, and high octenol release with CO_2. The high-level octenol release rate was chosen because it seemed to have a slight attractant effect for *C. sonorensis*. Release rates of octenol alone were 13.3 ± 5.0 mg/h and release rates of octenol with CO_2 were 11.5 ± 4.18 mg/h. Carbon dioxide was supplied by filling the paint cans with approximately 1.4 kg of broken dry ice. Holes in the containers allowed CO_2 gas to escape. Cans were weighed with dry ice before placement in the field and just after removal after a night's sublimation and had lost an average of 835 ± 174 g. Based on earlier studies, the gas release rate around sunset, when host-seeking activity should have been highest, should have been approximately 1,000 ml/min (Mullens 1995).

Collections from the initial trials (octenol without CO_2) were low and were subjected to chi-square analysis. Collections from the 2nd series (octenol alone, CO_2 alone, and the combination) were subjected to analysis of variance, using treatment, block, and interaction effects and Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$) to separate means.

Repellent studies: The repellent studies were conducted between September 16 and October 22,

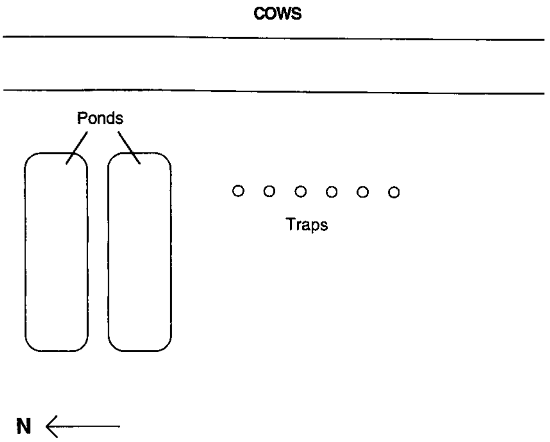

Fig. 2. Diagram (not exact scale) of suction trap locations at CO dairy used for *Culicoides sonorensis* collecting employing potential repellents. Traps were separated by 6.6 m.

1997, at the CO dairy farm in the same area of western Riverside County, CA. An open pasture at the western edge of the dairy was used to deploy a row of 6 traps that were 6.6 m apart (Fig. 2). The traps were 30 m west of pens where dairy cows were held and at least 41 m south of 2 wastewater ponds that provided developmental sites for *C. sonorensis*. Traps were set 30 min before sunset and were operated for 4 h. After each hour of operation the catch bags were replaced; the following day hourly trap catches were counted.

The following preparations were tested: a proprietary, plant-derived (Meliaceae) extract with 4.5% of active ingredient (AI), code-named Ag1000, with 2-heptanone as a solvent; another proprietary product called Lice Free, which contains 2% AI from plant extracts in water (Eldar Cosmetic Industries Ltd., Nes Ziyyona, Israel); Mosi-guard with 50% *Eucalyptus maculata* var. *citriodora* Hook extract, with isopropyl alcohol as a solvent (Medical Advisory Services for Travellers Abroad, Ltd., Keppel Street, London, United Kingdom); deet, 15% (Aldrich Chemical Co., Milwaukee, WI); 2-heptanone (Sigma, St. Louis, MO); and untreated control.

The method of repellent-impregnated bed nets, as recommended by the World Health Organization (Schreck and Self 1985) and modified by Braverman and Chizov-Ginzburg (1997) was used. Portable suction light traps as used in the octenol studies, but with the light bulbs in place, were modified by adding a 24-cm-diameter plastic funnel to the top entrance of the suction trap. The light bulb thus was positioned at the base of the funnel (Fig. 3). Circular nets with mesh size #10 (each hole 1.9 × 1.9 mm) made of 50% cotton and 50% polyester were clipped to the upper rims of the funnels after treatment (see below).

Nets had a radius of 17.46 cm and a surface area of 957 cm^2 (0.09 m^2). The pieces of nets were weighed, then immersed for 30 min in the various repellents or their solvents. Nets then were dried for 30 min and weighed, as described by Braverman and Chizov-Ginzburg (1997). Nets were transported to and from the field site inside sealed plastic bags. The rounded pieces of impregnated nets were fitted with clamps on the entrance of each trap. The size of the net's holes allowed free access of the *C. sonorensis* midges to the light source of the trap. Each net was used only once, for 1 experimental preparation. The amount of preparation absorbed was calculated per m^2 of net (Table 1), according to the World Health Organization method (Schreck and Self 1985).

The 6 treatments initially were randomized and then rotated for 6 nights in a Latin-square design (each treatment occupied each position for 1 night). Analyses of variance tests were performed on the numbers of females collected using the general linear model procedure (SAS Institute 1990), using night, time, position, and treatments as independent variables, plus the treatment × time interaction. Early evening winds often reduced collections for part or all of the 1st hour of trap deployment. For the analysis, hourly collections were grouped into 2 time periods (1st 2 h and last 2 h). Before analysis raw means were subjected to a square root ($n + 1$) transformation to stabilize the variance. Transformed means were separated using Tukey's HSD test with an alpha level of 0.05. Raw means are presented.

RESULTS

Octenol trials

Octenol trials were done at the AK dairy during a period of high *C. sonorensis* activity in the area, but numbers of insects collected in the 1st series of experiments were consistently very low (average < 15 males or females/trap). This made parametric comparisons difficult. However, χ^2 comparisons of numbers taken over all 6 nights in the octenol versus no-bait traps suggested a very minor, but statistically significant, attraction of both females and males to octenol alone (Table 1). Collections were somewhat higher in the high octenol treatment than in the low-octenol treatment for both sexes.

Subsequent trials using octenol alone, CO_2 alone, and the combination demonstrated that traps using CO_2 collected far more males and females ($P < 0.01$) than did traps baited with octenol alone (Table 2). Further, addition of octenol to the CO_2 did not significantly affect trap collections relative to CO_2 alone.

Repellent trials

Numbers of *C. sonorensis* females taken in traps with different potential repellent treatments are pre-

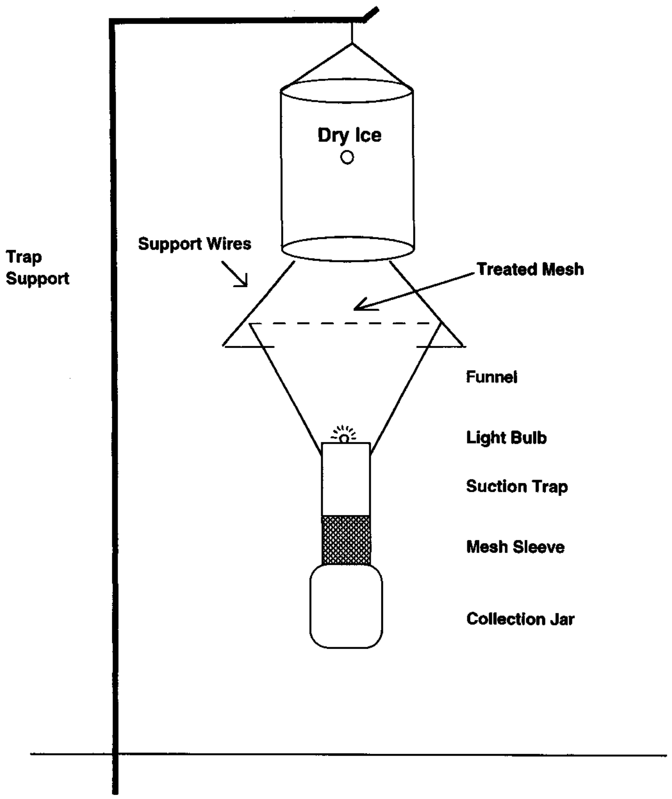

Fig. 3. Diagram of suction light trap used in conjunction with CO_2 and repellent-treated mesh for collecting *Culicoides sonorensis.*

sented in Fig. 4. Too few males were collected for analysis. The regression model explained 54% of the variability in the data set. Night and position effects were highly significant ($P < 0.01$); collections were higher early in the study period and closer to the pond. However, time effects were not significant ($P > 0.1$) and no time–treatment interaction was found. Taking other independent variables into account, treatment effects still were significant ($P = 0.017$). Relative to the untreated control, collections in the heptanone trap were slightly but not significantly higher (+27%). Although not significantly different from the control, collections in the traps with the lice repellent, Ag1000, and Mosiguard were reduced by 15, 34, and 39%, respectively. Collections in the trap with deet were significantly (66%) lower than the heptanone ($P < 0.05$) and 56% less than the untreated control ($P > 0.05$).

DISCUSSION

In contrast to studies on other *Culicoides* species (Takken and Kline 1989; Kline et al. 1994; Ritchie et al. 1994; Blackwell et al. 1996, 1997), this study did not show any substantial attraction of *C. sonorensis* to this level of octenol or synergism of CO_2 by octenol. Cattle are substantially more attractive to *C. sonorensis* than is explained by their CO_2 output alone (Mullens and Gerry 1998), and this species readily feeds on bovids, which produce octenol (Hall et al. 1984). Therefore, it is somewhat surprising that *C. sonorensis* showed so little response to octenol. Conducting the experiments fairly close

Table 1. *Culicoides sonorensis* midges trapped in 6 nights at AK farm with low and high octenol rates, versus unbaited control. Percentages of total are shown; total number is noted. Analysis was done on raw counts.

Sex	Control	Octenol (mg/h) Low (1.2 mg)	Octenol (mg/h) High (11.5 mg)	Total no.	χ^2	P[1]
F	22.6%	34.6%	42.7%	164	10.05	<0.01
M	23.9%	29.3%	46.2%	184	14.49	<0.01

[1] Testing the hypothesis that treatments do not affect collections.

Table 2. Numbers of *Culicoides sonorensis* midges (mean ± SD) trapped in 4 nights at AK farm with CO_2 alone, octenol alone (11–14 mg/h), and CO_2 together with octenol.[1]

	Octenol alone	CO_2 alone	CO_2 + octenol
Females	5.1 ± 3.8b	353.2 ± 230.2a	378.9 ± 211.0a
Males	19.5 ± 18.7b	65.0 ± 41.1a	65.9 ± 45.7a

[1] Values within rows (by sex) followed by different letters are significantly different ($P > 0.01$); Tukey's honestly significant difference test.

to the animals, and resulting background levels of octenol in the air, possibly minimized the effect of octenol released from our traps. Alternatively, this level of octenol may have been too high (or even too low), although it is in the range (4–41 mg/h) that is attractive or synergistic with CO_2 for some mosquito species in the field (Kline et al. 1991). The octenol rate used in the present studies was higher than that used in some other *Culicoides* studies. For example, *C. impunctatus* was extremely sensitive to octenol (0.1 mg/day) (Blackwell et al. 1996). Other field trials have shown positive results for *Culicoides* using a release rate of 1.6–3.0 mg/h (Takken and Kline 1989, Kline et al. 1990). However Ritchie et al. (1994) noted synergism of CO_2 by octenol at release rates of 6 and 29 mg/h, depending on the species of *Culicoides.* However, CO_2 still was a potent attractant despite proximity to the cattle. Evidently other semiochemicals, their manner of release, or physical or visual cues are important in orientation of *C. sonorensis* to hosts. The collection of males in the CO_2-baited traps is consistent with recent work demonstrating attraction of *C. sonorensis* males to CO_2-baited suction traps and to hosts (Gerry and Mullens 1998, Mullens and Gerry 1998).

The repellent trials were done later in the season when adult *C. sonorensis* activity had diminished. Despite substantial variability among nights and positions, the Latin-square design allowed treatment effects to be detected. The method of field screening of potential repellents or attractants using the combination of treated mesh traps seems to have promise. The trend toward activity of Mosiguard, for example, agrees with laboratory studies on *C. sonorensis* (Trigg and Hill 1996), and activity of deet also was expected because of activity against *C. impunctatus* (Trigg 1996) and other *Culicoides* species (see Magnon et al. 1991). Interestingly, time effects were not significant, as was the case with treatment–time interaction. This implied that the effects of the materials did not vary substantially over the 4-h deployment period. However, results were influenced by winds, which varied in intensity and duration among nights. The testing method was a modification of that of Braverman and Chizov-Ginzburg (1997), but instead of using a suction trap equipped with a black light source inside an animal pen, a smaller, battery-powered suction light trap with dry ice was used. Superficially, this trap uses the same principle, but the repellent-impregnated nets were cooled by the dry

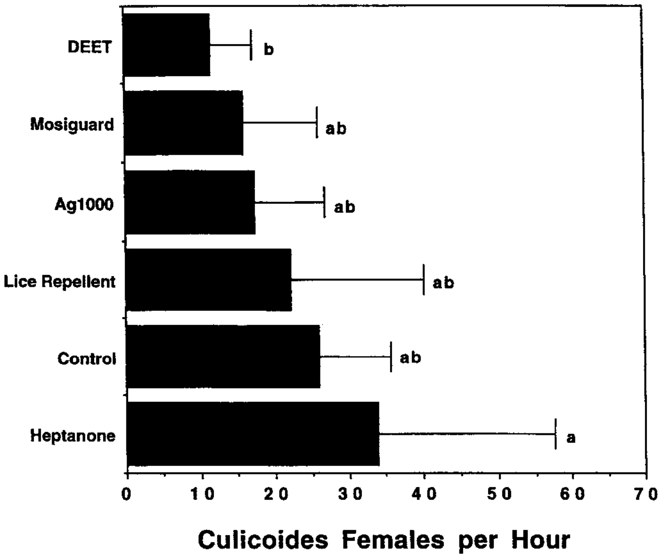

Fig. 4. Average (±SE) numbers of *Culicoides sonorensis* females collected in suction light traps with CO_2 and repellent-treated mesh over 6 evenings.

ice, which caused water to condense on the nets late in the season. These factors reduced the evaporation of the repellents from the nets and probably distorted the actual repellency effect. In future work, use of CO_2 from tanks would be preferable. Although lacking the control of an olfactometer, hourly testing of potentially repellent compounds with impregnated nets in the field has some advantage, in that it more closely reflects complex field settings. Thus, this technique might be a valuable intermediate step between olfactometer trials and trials using live hosts in the field. This technique also may allow initial work with materials not yet ready for application to vertebrates, species not amenable to laboratory manipulation, or species not available from laboratory colonies.

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